

## Contributed Mini Review

## The potential of mesenchymal stem cells derived from amniotic membrane and amniotic fluid for neuronal regenerative therapy

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The mesenchymal stem cells (MSCs), which are derived from the mesoderm, are considered as a readily available source for tissue engineering. They have multipotent differentiation capacity and can be differentiated into various cell types. Many studies have demonstrated that the MSCs identified from amniotic membrane (AM-MSCs) and amniotic fluid (AF-MSCs) are shows advantages for many reasons, including the possibility of noninvasive isolation, multipotency, self-renewal, low immunogenicity, anti-inflammatory and nontumorigenicity properties, and minimal ethical problem. The AF-MSCs and AM-MSCs may be appropriate sources of mesenchymal stem cells for regenerative medicine, as an alternative to embryonic stem cells (ESCs). Recently, regenerative treatments such as tissue engineering and cell transplantation have shown potential in clinical applications for degenerative diseases. Therefore, amnion and MSCs derived from amnion can be applied to cell therapy in neuro-degeneration diseases. In this review, we will describe the potential of AM-MSCs and AF-MSCs, with particular focus on cures for neuronal degenerative diseases. [BMB Reports 2014; 47(3): 135-140]

## INTRODUCTION

As tissue engineering has developed, cell-based therapy has emerged in clinical trials due to potential applications in regenerative medicine. Tissue engineering using stem cells has introduced a new field of repair in the treatment of degenerative tissues or diseases.

The central nervous system (CNS), is typically an unrecoverable site, that it, it cannot be completely regenerated after

damaged (1). Therefore, most degenerative neurological diseases and mechanical and physical injuries remain incurable. To improve the treatment of neurodegenerative disorders, stem cell-based regenerative therapy has been investigated. Moreover, the various types of stem cells include embryonic stem cells (ESCs), adult stem cells, mesenchymal stem cells (MSCs), and induced pluripotent stem (iPS) cells have been considered as possible sources of regenerative treatment.

MSCs, generally from adult stem cells, are derived from the mesoderm and identified available in mammal tissues, such as bone marrow, adipose, umbilical cord blood, olfactory bulb, amnion, and Wharton's jelly (2, 3). MSCs can be multipotent and differentiated into various cell types, not only mesenchymal lineage cells, such as chondrocytes, adipocytes, osteocytes, but also nonmesodermal lineage, such as epithelial cells, hepatocyte cells, cardiomyocyte cells, insulin secreting pancreatic  $\beta$ -cells, and neuronal cells (4-9). In addition, MSCs have several advantages, including their nontumorigenicity and anti-immunogenic activity and minimal ethical problems compared with any other candidates for regenerative therapy. These facts suggest that MSCs are considered a readily available source for regenerative medicine.

The MSCs derived from amniotic membrane (AM-MSCs) and amniotic fluid (AF-MSCs) have been reported as a better new prospective field of regenerative medicine compared with other MSCs sources, because of the easily of their acquisition, reduced donor damage, multipotency, low immune response, and acceptable ethical issue (10).

In this review, we will describe the potential of the AF-MSCs and AM-MSCs in tissue engineering and cell transplantation strategies for curing degenerative neuronal diseases.

**Embryonic stem cells (ESCs) vs. induced pluripotent stem (iPS) cells**

The research on ESCs began with the establishment of mouse ESCs lines in 1981, and it advanced with the first cultivation of human ESCs derived from blastocyst in 1998 (11, 12). Commonly, ESCs are capable of long-term self-renewal and maintaining a continuously undifferentiated state, and they have the pluripotent differentiation ability (13). With these characteristics, ESCs can be considered as significant biomedical materials for

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developmental biology and regenerative medicine.

However, despite the outstanding potential of ESCs, they still have some drawbacks for clinical use. Many experimental studies observed tumor formation after injection of ESCs for treating injury (14). Moreover, this tumor formation with the immunogenicity lead to safety concerns, and thus the modification of ESCs of cell-based therapies has difficulties. In addition to the safety concerns involved in ESCs-based therapy, ethical concerns are another issue. The moral debate surrounding testing to discover the potential of ESCs continues.

The iPS cells are a type of pluripotent stem cell, which are artificially induced by inducing a few specific transcription factors into nonstem cells. The reprogrammed cells also differentiate into various cell types including the three major cell types, both *in vivo* and *in vitro* (15). These established iPS cells are pluripotent stem cells, which eliminates the concern of immune rejection, and they can be used for the patient-specific transplantation therapy.

However, using the viral transfection system creates concern for clinical applications. This viral genome integration into the host genome might increase the risk for formation of teratoma (16). In the reprogramming process, the insertion of genes randomly located in the host cell's genome results in unstable expression of the pluripotent genes and chromosomes. Thus, a reprogramming technique for safe iPS cells should be developed for cell therapies with iPS cells.

#### Amniotic-membrane-derived mesenchymal stem cells

The amniotic membrane is a component of the placenta that originates in the extra-embryonic tissue and functions to protect the fetus during pregnancy with supplemental nutrients. The amniotic membrane is generally known as bio-material for

treatment scaffolds of burns, skin, and corneal transplantation, because it has the ability to scarring reduction and anti-inflammatory properties (17). Currently, the amniotic membrane is widely used as material for clinical treatment (Table 1).

The AM-MSCs can differentiate into all three germ layers for ectodermal lineage cells, mesodermal lineage cells, and endodermal lineage cells (18). They are positively expressed mesenchymal markers, such as CD105 and CD90, and negatively expressed hematopoietic markers, such as CD29, CD34, CD45, and CD105 (19). In addition, the amniotic membrane expresses anti-angiogenic and anti-inflammatory proteins, and does not express human leukocyte antigen (HLA)-A, -B, and DR antigens (20). These results show that the AM-MSCs are very important for advanced regenerative medicine, because inflammatory and immunogenicity remain indispensable factors in successful transplantation.

Moreover, despite the pluripotent marker expression of AM-MSCs, such as Oct-4, Nanog, TRA-1-60, and TRA-1-81, they do not form teratoma (21). In addition, the native amniotic membrane expresses many types of growth factors, and these growth factors reduce inflammation and prevent fibrosis induced by inflammation (22). Further, amniotic membrane has no blood vessels or nerves, and it enhances wound healing by inhibition of proteinase activity (23). Therefore, immunological rejection after transplantation does not occur in the amniotic membrane and the cells derived from it. For these reasons, amniotic membrane and AM-MSCs might be useful sources for transplantation for regenerative disease treatment.

#### Amniotic fluid-derived mesenchymal stem cells

The amniotic fluid was formed at 2 weeks after fertilization in the amniotic cavity of early gestation. Amniotic fluid is important to keep the fetus safe, and it supports organ development. The first progenitor cells derived from amniotic fluid was reported in 1993 by Torricelli *et al.* (24). The amniotic fluids incorporated a heterogeneous population of cells of fetal origin, and these cells maintained fetal-specific alleles without loss of the chromosomal telomere length in 250 doubling times (25).

Recently, many studies have identified amniotic fluid (AF) as a new source of MSCs, and these AF-MSCs express the pluripotent marker Oct-4 in almost 90% of the active condition, and they also have multiple differentiation capacity like the AM-MSCs (26, 27). Further, AF is routinely used to perform the standard evaluation of karyotyping and they are genetic and molecular tested for diagnostic purposes. After prenatal diagnostic testing, AF cells can be used as a source of fetal progenitor cells or otherwise discarded (28). Use of these cells could minimize the ethical objections, have a high renewal activity, and maintain stability. The AF-MSCs enable the uses of autologous cells obtained from patients' tissues. They can be easily isolated. Moreover, they maintain genetic stability and offer advantages of nontumorigenicity, low immunogenic activity, and present minimal ethical issues. These findings show that AF-MSCs are being considered as potential sources of treat-

**Table 1.** Current state of therapeutic potential in amniotic membrane

Target tissue	Disease	Application	Ref.
Eye	Burn	Surgical dressing, burn treatment, tissue graft	(23)
	Ocular surface disease	Tissue graft	(17, 39)
Skin	Burn	Tissue graft, moist wound dressing	(40)
Oral cavity	Tympanoplasty, Vestibuloplasty	Tissue graft, surgical dressing	(41, 42)
Abdomen	Gastroschisis	Used for closure material	(43)
$\beta$ -cell	Type-1 diabetes	Patient newborn child's cell injection	(44)
Bone	Postnatal sternal repair	Engineered osseous	(45)
CNS	Spinal cord injury	Preclinical animal studies, tissue graft	(46, 47)
Vagina	Myelomeningocele	Autograft	(48)
	MRKH syndrome	Vaginoplasty	(49)

MRKH: mayer-rokitansky-küster-hauser syndrome.

**Table 2.** Clinical application of amniotic fluid

Target tissue	Disease	Application	Ref.
Fetus	Fetal abnormality	Biochemical tests, prenatal diagnosis	(50)
Skin	Wound	Tissue graft	(51)
Heart	Cardiac malformation	Autologous heart valve tissue engineering	(52)
$\beta$ -cell	Type-1 diabetes	Preclinical animal studies, cell transplantation	(53)
CNS	Ischemic stroke	Preclinical animal studies, intravenous cell grafts	(54)
Limb	Hind limb ischemia	Preclinical animal studies, cell injection, angiogenesis	(55)
	Foot and ankle wounds	Allograft	(56)

ment by many researches and clinical application (Table 2).

#### Application for neuronal regenerative therapy

Despite the inability to regenerate injured CNSs, research focused on repairing damage resulting from neurodegenerative diseases is a public interest (29). As research continues, the clinical trials of stem cell-mediated neuronal regeneration therapy are being announced. Many of MSC-type cell studies have reported that MSCs have the ability of differentiated into neural-like cells *in vitro* and they have a long history as potential sources for neural differentiation studies (30-32).

Recent research has confirmed that the MSCs can be remyelinated in models of demyelination that do not involved inflammation (33). The amniotic membrane and AF have been noted as new alternative sources of MSCs that would be useful for clinical applications (Table 3). In the undifferentiated condition, AM-MSCs and AF-MSCs possess characteristics primed for neural differentiation by express the neuron and neurotransmitter factors (34). Moreover, they are expressed neurotrophic factors, and these factors lead to bio delivery and enhance the protection and promote the regeneration of damaged tissue (35).

Further, recent research has reported that dopamine and tyrosine hydroxylase (TH) enzyme weakly express undifferentiated amnion, and they are ready for use as potential sources for the induction of functional dopaminergic neurons (36). Further, the therapeutic effects of AM-MSCs and AF-MSCs can significantly improve the motor functions of Parkinson's diseases models (37). Thus, amnion-derived stem cells have the potential to be differentiated into dopaminergic neurons.

From these data, we hypothesized that AM-MSCs and AF-MSCs could be attractive engraft materials of differentiation neuronal-like cells and developing cell-based treatments for various neuronal degenerative disorders.

**Table 3.** Summary of potential property of amniotic membrane and fluid in neuronal regeneration therapy

Field	Potential property	Ref.
Differentiation	Neuronal precursor cell,	(57)
	Dopaminergic neuronal cell	(58)
Transplantation	Alzheimer's disease model ; Improved spatial memory	(59)
	Parkinson's disease ; Functional improvement	(60)
	Peripheral Nerve Regeneration	(61)
	Focal cerebral ischemia-reperfusion injury	(62)
	Sciatic nerve crush injury	(63)
	Spinal cord injury	(64)

## DISCUSSION

The limitation of the regenerative properties of the CNS is that it can be seriously life-threatening. In the world report, the number of patients with Alzheimer's, the most common neurological disorder, is estimated at 36 million people worldwide, and Parkinson's disease affects 1% of the population above the age of 60 (38). A much more progressive approach of therapeutic research has suggested the possibility that stem cells may have therapeutic effects for various neurological diseases.

Stem cell-based therapeutic strategies are showing potential in experimental studies. However, some problems of stem cells including safety and ethical issues have limited their clinical use. In addition, some MSCs also have safety problems and thus clinical application of treatment is engendered. Thus, the amniotic membrane and fluid are considered as non-controversial sources because of the use of either heterologous ESCs or the less ethically disputed MSCs.

Compared to other stem cells, amniotic cells can be easily collected during routine prenatal testing, and the amniotic membrane can also be obtained during cesarean section after birth. These isolation methods are noninvasive progress without destroying human embryos and thus alleviate the ethical controversy. A small amount of AF obtained by amniocentesis and amniotic membrane samples could produce enough MSCs for applications of tissue engineering. Further, these MSCs secrete trophic factors that may be neuro-protection and may promote nerve regeneration after being transplanted into an injured neuron or neuronal site.

As needed, AM-MSCs and AF-MSCs could be stored and provided immediately for future autologous therapy. The autologous tissues made from patient-specific cells could be applied to nonrejected transplantation. Further, studies of AM-MSCs and AF-MSCs demonstrate the prospects of potential therapeutic uses for several CNS diseases. It is hoped that these beneficial effects of AM-MSCs and AF-MSCs will gradually be develop into therapeutic outcomes for neural regeneration.

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